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# Evaluation of the EraGen MultiCode®-PLx Respiratory Virus Panel using Barcoded Magnetic Beads designed to detect seventeen respiratory viruses

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## OBJECTIVE

To evaluate the performance characteristics of EraGen Biosciences' MultiCode®-PLx Respiratory Virus Panel (RVP) using Applied BioCode's Barcoded Magnetic Beads (BMB) and the BioCode Analyzer.

## METHODS

**Specimens:** A total of 164 retrospective respiratory specimens (134 positive and 30 negative) from 2009-2010 were tested in triplicate with MultiCode®-PLx RVP using Applied BioCode's BMB and the BioCode analyzer.

**Reference Methods:** The reference method for 15 of the 17 viruses (RSV A, RSV B, PIV1, PIV2, PIV3, PIV4a, PIV4b, hMPV, HRV, CoV-OC43, CoV-NL63, CoV-229E, AdB, AdC, and AdE) was the EraGen MultiCode®-PLx RVP using the Luminex detection platform. The reference method for Influenza A & B was the CDC IVD Influenza RTPCR assay.

**Nucleic Acid (NA) Extraction:** Nucleic acids were isolated from clinical specimens using Roche MagNA Pure Total Nucleic Acid Kit with an input & output volumes of 100 µL for each specimen.

**Reverse Transcription (RT) and Target Amplification:** Nucleic acid from the clinical specimens was reverse transcribed using random hexamers and AMV reverse transcriptase. The cDNA from the RT step was amplified using target specific primer sets via PCR. For each viral target, one target-specific primer contained a single iC at the 5' end for subsequent site-specific labeling with diGTP-biotin. [Fig 1A]

**Target-Specific Extension (TSE):** TSE reactions were performed on PCR amplified products using target-specific extenders to generate diGTP-biotin labeled PCR amplified products. [Fig 1A]. Target-specific extenders contain 3' bases that are target specific and 5' bases that are complementary to the EraCodes.

**Barcoded Magnetic Beads (BMB):** Barcoded Magnetic Beads are 100 X 30 x 6 µm beads formed by combining biocompatible polymer with paramagnetic material. These optically bar-coded beads are functionalized with carboxyl groups on the surface for conjugation to amine-containing EraCodes. The BMB barcode patterns provide a high contrast transmitted signal allowing the barcode to be decoded and the bead identified.

**Capture and Detection of Labeled BMBs:** Biotin labeled TSE products were captured using EraCode coupled BMB via a hybridization reaction at room temperature and detected using Streptavidin-Phycoerythrin (SA-PE) [Fig 1B]. The beads were washed, re-suspended in Detection buffer and analyzed using the BioCode-1000 analyzer. [Fig.1C]. The BMB were decoded and the associated fluorescence emitting from the BMBs were expressed as Median Fluorescence Intensity (MFI) values. A MFI value of 1000 was used as the cutoff threshold and samples wherein the MFI was greater than the threshold for at least 2 of the 3 replicates were scored as positives.

**Discordant Analysis:** Specimens with discordant results were resolved by reextracting the original specimen and testing with both the RVP assay using the BMB and the reference method.

**Reproducibility:** The assay was performed in triplicate by two microbiologists on separate days.

MultiCode®-PLx Technology

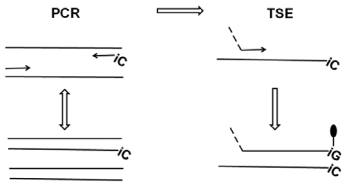



**Fig.1A: MultiCode-PLx PCR & TSE**

Barcoded Magnetic Beads (BMB)

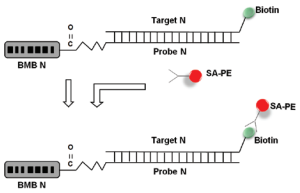
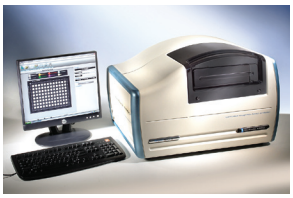



**Fig.1B: Capture and Detection of BMBs**

Applied BioCode Analyzer



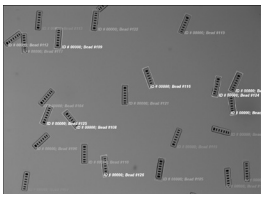


**Fig.1C: BioCode-1000 Analyzer**

## RESULTS

- MultiCode®-PLx RVP on Applied BioCode platform was 100% sensitive for eleven respiratory viruses and 92.9% sensitive for Adenovirus B (n=13) [Fig. 2].
- Sensitivity varied for influenza viruses:
  - Influenza B (n=10) and seasonal A H3 (n=10) were 100% sensitive.
  - Influenza 2009 H1N1 was 62.5% sensitive (n=10).
- Specificity of the RVP assay using BMB was 100% for all seventeen of the respiratory viruses included in the panel.
- No data were available for Coronaviruses 229E and NL63 or for Parainfluenza 4a or 4b due to the lack of positive specimens.

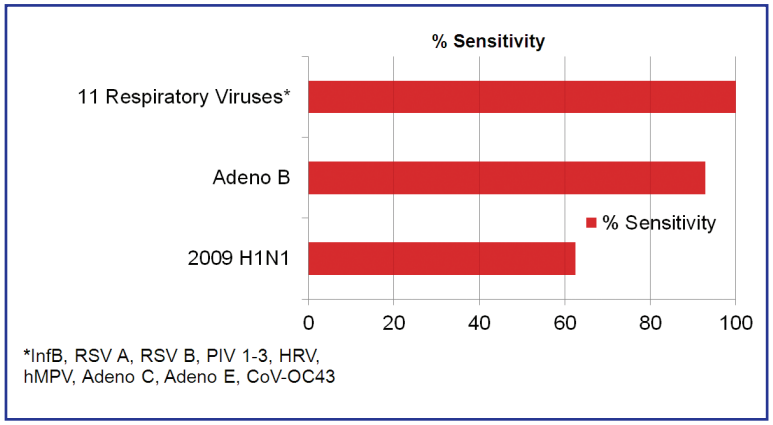



**Fig. 2: Sensitivity of the MultiCode®-PLx RVP assay**

- Overall, the MultiCode®-PLx RVP using the BMB was 92.5% sensitive for all viruses tested (n=134).
- Excluding Influenza A, there was >99% agreement between the Applied BioCode and the Luminex detection platforms.
- Eighteen of the 134 (4.5%) specimens tested in triplicate produced uninterpretable results due to high background MFI resulting from incomplete removal of SA-PE.
- There was a clear distinction between MFI values for positive and negative specimens [Fig.3].

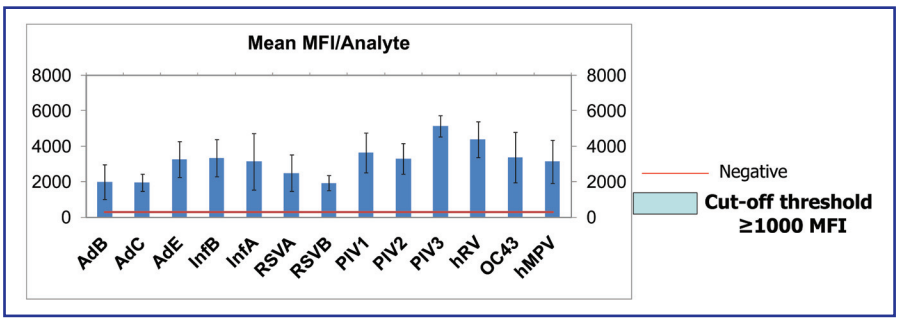



**Fig. 3: MFI values for positive and negative specimens**

## DISCUSSION

- The lower sensitivity for 2009 H1N1 observed in this study is due to the fact that the Influenza design pre-dates the 2009 H1N1 pandemic and the sequencing data of the 2009 H1N1 samples showed that the decreased sensitivity was due to primer-target mismatches.
- The Flu A primers were redesigned and the 10 clinical specimens were retested using MultiCode®-PLx RVP.
- All of the 10 specimens were detected with the redesigned Flu A primer [Table 1].
- The sensitivity of this assay for Flu A with the redesigned primers was 100%.
- Further research is needed to determine whether Flu A primers will detect emerging Influenza virus strains.

**Table 1: Results of MultiCode®-PLx RVP assay using redesigned (2011) Flu A primers**

| Specimen Type | Reference Result | Existing Flu A Design | Redesigned Flu A |
|---|---|---|---|
| NP | FluA 09 H1N1 | 0/3 | 3/3 |
| not given | FluA 09 H1N1 | 1/3 | 3/3 |
| NP | FluA 09 H1N1 | 0/3 | 3/3 |
| NP | FluA 09 H1N1 | 3/3 | 3/3 |
| NP | FluA 09 H1N1 | 3/3 | 3/3 |
| NP | FluA 09 H1N1 | 2/3 | 3/3 |
| not given | FluA 09 H1N1 | 0/3 | 3/3 |
| NP | FluA 09 H1N1 | 0/3 | 3/3 |
| NP | FluA 09 H1N1 | 3/3 | 3/3 |
| not given | FluA 09 H1N1 | 0/3 | 3/3 |

**Each specimen was tested with both designs and the number of positives out of 3 replicates is shown [EraGen Biosciences].**

- The use of an automated plate washer will improve consistency of residual SA-PE removal and likely remedy the high background seen with a few of the specimens.
- Decreased sensitivity observed for Adenovirus B may have been due to low virus titer or degradation of the sample from freeze-thaw cycles.

## CONCLUSIONS

- The sensitivity of the RVP assay using BMB was comparable to the MultiCode®–PLx RVP on the Luminex 100 IS TM platform.
- The Flu A primers designed prior to 2009 had low sensitivity for 2009 H1N1 and was therefore redesigned and significantly improved detection of the 2009 H1N1 virus.
- RVP test results for all 17 targets are available in <8 hours.
- The RVP assay is useful for respiratory virus surveillance and outbreak response.
- Enhances capability to detect co-infections with multiple viruses.
- The Applied BioCode platform offers advantages such as the ability to re-read test runs, fewer reagents, and requires less routine maintenance.
- The MultiCode®-PLx RVP assay using BMB and the Applied BioCode detection platform proved to be a viable alternative to the Luminex system and offers a high degree of sensitivity, specificity and reproducibility.

## ACKNOWLEDGEMENTS

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